OBSERVATIONS ON *HYDRA* AND *PELMATOHYDRA* UNDER DETERMINED HYDROGEN ION CONCENTRATION.

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Much has been written recently concerning reduction, de-differentiation and resorption in *Hydra*. It is generally conceded that reduction in hydra is accompanied by a loss of tentacles. The literature enumerates the following causes by which hydras lose their tentacles. N. Annandale ('07) observed, in studying *Hydra orientalis*, that during the hot season of the year this species has but four tentacles while during the cold season it has six tentacles. G. Entz ('12) observed that an infection with *Amœba hydroxena* may lead to a degeneration of tentacles. Reynolds and Looper ('28) have come to the conclusion that this parasite is responsible for the degeneration of the tentacles. Certain ciliates recorded by E. Reukauf ('12) and P. Shultze ('13) also caused the loss of tentacles. E. Shultz ('06) observed that hunger set up a process of dedifferentiation within the tentacles. Huxley and DeBeer ('23) observed that adverse environmental conditions accelerate dedifferentiation and resorption of the tentacles of *Obelia* and *Campanularia*. They also found that this process of dedifferentiation and resorption might involve not only the tentacles but also part of the zoöid. Berninger ('10) found that, in response to inanition, hydra lost its tentacles. Finally Kepner and Jester ('27) also observed that the loss of tentacles was brought about in response to inanition. This loss, according to them, was accomplished by ingestion of the tips of the tentacles through the mouth. This may occur, but undoubtedly is not the usual method, as Hyman ('28) indicated.

It is a well known fact that the concentration of the hydrogen ion medium that bathes the protoplasm or protoplasmic tissue

has a profound effect upon it, therefore it seems strange that no attempts have been made to account for reduction, dedifferentiation and resorption on the basis of such environmental conditions. The following observations and results have been obtained through an effort to determine whether or not the concentration of the hydrogen ion is an important factor with reference to the three above mentioned phenomena.

METHODS AND MATERIALS.

Filtered spring water in 300 cc. portions kept in thoroughly cleansed glass dishes was used as a culture medium.

Very dilute solutions of $N/20$ sodium hydroxide and of hydrochloric acid were used in quantities to adjust the pH of the solutions. The colorimeter method was used for the pH determination of the solutions and LaMotte color standards were employed for color matching. Tests, adjustments and observations were made every twenty-four hours except where otherwise indicated. The temperature was maintained between 18 and 22° C. During these investigations frequent examinations were made of both the culture and of the animals for protozoa which might have been responsible for reduction. None were found except where stated. Observations were made with a dissecting binocular of a magnification of twenty diameters. These observations were supplemented by histological preparations.

At first distilled water was tried as a culture medium with the idea that a more accurate determination could be made of the hydrogen ion concentration. Various deleterious factors enter into the use of such a medium so it was discarded. In the subsequent experiments, filtered spring water was used.

The terms reduction, dedifferentiation and resorption, as used by other authors and us, may be defined as follows: Reduction is a uniform decrease in surface area in which process the ectoderm, mesoglea and endoderm remain intact and maintain a normal position in relation to each other. Dedifferentiation and resorption represent a dual phenomenon which involves a local reduction of surface. The presence of this dual phenomenon in the tentacles is indicated by a thickening and knobbed appearance at the tips of the tentacles.

EXPERIMENTAL.

Culture 1.—Four *Pelmatohydra oligactis* (Pallas), were placed in a culture medium consisting of distilled water and NaOH was added to maintain a constant pH of 7.8. At the end of a period of six days there was much apparent reduction and resorption of the tentacles in all specimens. One polyp was fed on the sixth day and one on the seventh. At this point the experiment was terminated through an accident.

Culture 2.—Four *Pelmatohydra oligactis* were placed in a culture medium of distilled water. The culture maintained a pH of 6.8 without the addition of either hydrogen or hydroxyl ions. These polyps disintegrated in five days.

Culture 3.—Four *Pelmatohydra oligactis* were placed in a culture medium consisting of distilled water. This culture maintained a pH of 7.0 which was fatal to the polyps in five days. At this phase of our observations we came to the conclusion that we were imposing other factors than the controlled pH represented, upon the hydras in using distilled water. A change in osmotic pressure was undoubtedly involved when distilled water was used instead of spring water. So, from this point on, spring water was employed as the medium in which to keep the observed polyps.

Culture 4.—Four *Chlorohydra viridissima* (Pallas) were taken from spring water which tested pH 7.6. They were normal in every respect. The pH of the second lot of spring water was now maintained at 6.6. The only change being made here was using a second glass dish similar to the one in which the pH tested 7.6 and in the pH now being 6.6. In five days, six of the polyps had disintegrated and the remaining one had undergone advanced dedifferentiation and resorption. It was placed in filtered spring water of pH 8.6 in an effort to bring about regeneration but it disintegrated in a few hours. This result, together with general observations made on various cultures, in the laboratory, in which the polyps displayed marked dedifferentiation and resorption, indicates that the acid condition of the medium induces dedifferentiation and resorption. Our observation upon a lower hydrogen ion concentration (higher pH) proved to be little more instructive as seen by the following culture.

Culture 5.—Six *Pelmatogydra oligactis* were isolated in filtered spring water the pH of which was maintained between 7.6 and 8.2. On the 8th day all of the hydras appeared perfectly normal; however, on the 9th day, all except one had disintegrated. The one remaining hydra showed no apparent reduction or de-differentiation and resorption of the tentacles. This hydra was sectioned and its histology appears later in the paper.

On several occasions similar results were obtained when the pH was held within the range from pH 7.8–8.0. It appears that the first ten days represent a critical period when the polyps are exposed to inanition. After the 10th day has passed we have had uniform results as the following observations indicate.

Culture 6.—Four *Chlorohydra viridissima*, in which some re-sorption was displayed, were isolated in filtered spring water pH 6.6. This water was over *Elodea* which had been previously boiled. The *Elodea* was separated from the polyps by a double thickness of cheese-cloth spread over the bottom of the container. The *Elodea* was removed after six days and spring water alone was used. As indicated above, these hydras were in a somewhat re-sorbed condition. The pH of this culture was varied, first de-creasing the concentration of the hydrogen ions after the first two days up to 7.6, then increasing to 7.0, then again decreasing to 7.8. A pH of 7.8 was maintained for the last thirteen days. Immediately following these changes in pH, we observed the physiological aspect of the polyps. It was seen that the greater the concentration of the hydrogen ions the greater was the degree of dedifferentiation and resorption in the polyps. If the concen-tration of the hydrogen ions was lessened the hydras returned to normal. Two of the four hydras survived for a period of twenty-three days. One of these was sectioned (its histology is referred to later in the paper) and the other was lost during a transfer for examination. On the nineteenth day a green hydra, with much resorbed tentacles and bearing gonads, was introduced into this culture. In two days this hydra had gained its normal appear-ance but its gonads had partially disappeared. It was fed and placed in an aquarium containing food where it developed into a fine vegetative specimen apparently normal. In this last specimen the change from laboratory culture water to filtered spring water must have been a factor as well as the change in pH.

This does not however lessen the significance of the reaction of the other individuals of culture 6, wherein only the pH concentration has been the factor involved.

Culture 7.—Six *Chlorohydra viridissima* in a slightly resorbed condition were placed in filtered spring water without *Elodea* the pH of which tested 8.6. After the first two days the pH was maintained at 7.8 until this experiment was terminated. On the fourteenth day one hydra was sectioned. At the end of a period of twenty-four days three hydras remained. They were much reduced in size but their tentacles were apparently normal. On the twenty fifth day they were placed in an aquarium containing food where they lived for several days and attained nearly normal size. At this point our observations on these animals ceased.

These most interesting cases (cultures 6 and 7), in which the polyps that had been reduced and in which apparent dedifferentiation and resorption had taken place at a hydrogen ion concentration above the optimum, were restored to a completely normal condition when subjected to hydrogen ion concentration at or near the optimum. This undoubtedly indicates that food is not necessary for the regeneration of hydra, but regeneration depends rather upon the hydrogen ion concentration of the culture water. Kepner and Jester ('27) record one hydra which had lost all of its tentacles and without the presence of food the lost tentacles were replaced by regenerated ones in eight days. As the culture medium was frequently changed it is probable that a favorable pH was accidentally maintained. Hyman ('28) records the same phenomena when she says: "Depressed specimens may be caused to regenerate if the water is replaced by culture water" (page 78). Huxley and DeBeer in working with *Obelia* and *Campanularia* were unable to cause the regeneration of dedifferentiated and resorbed tissue.

Culture 8.—Eight *Pelmatohydra oligactis* were isolated in filtered spring water the pH of which was maintained for the first two days at 8.4 and for the remainder of the period it was kept at pH 7.8. On the tenth day three hydras had completely disintegrated without displaying reduction, dedifferentiation and resorption. On the 17th day, *Halteria* appeared in the culture. These were not abundant, about ten being found in the

field of the binocular dissecting microscope. As all the hydras appeared in the same condition one was sectioned. These sections showed no *Halteria* present within cœlenteron or the food vacuoles. But menatocysts were present in the epithelio-muscular cells of the endoderm and within the cœlenteron, hence the histology indicates that resorption had taken place. This resorption was so slight that it is overlooked by examination of the living polyps under a dissecting microscope. The culture medium was changed, so as to have water free of protozoa, and the observations continued. On the twenty third day one hydra was sectioned (its histology is referred to later). On the twenty fifth day the remaining three hydras were given bits of liver which they readily accepted. Thus indicating that they were not in a "depressed" condition as described by Hyman ('28). They were placed in an aquarium containing food where they were observed for several days. No indication of "depression" became evident during these observations nor was there any evidence of it at the time the observations ceased.

In order to determine wherein the optimum range of hydrogen ion concentration for the medium lay, both green and brown hydras were exposed to varying degree of hydrogen ion concentration ranging from pH 5.2–8.0 and the time recorded when all hydras had disappeared in each culture. The result of this experiment is given in the following table.

Four more cultures were run, with both green and brown hydras, one with a pH of 7.8, the other at pH 8.0. All the polyps in these cultures were alive at the end of a period of twenty four days.

This indicates that the optimum hydrogen ion concentration lies near pH 7.8. And further hydrogen ion concentration is an important factor in the determination of dedifferentiation and resorption; for, in the same medium (filtered spring water) with only the concentration of hydrogen and hydroxyl ions altered, we have been able to either induce or inhibit dedifferentiation and resorption. This does not support the later part of Hyman ('28) page 93, paragraph 2, Biological Bulletin volume LIV, January 1928, number 1 in her explanation of the phenomenon of depression when she says that "it is induced by transfer to clean fresh water." It is quite evident that, if two different lots of hydra

TABLE I.

The x Mark Indicates the Day of the Death of the Last Hydra in the Culture.

	Existence in Days.											
	2	3	4	5	6	7	8	9	10	11	12	13
Brown hydra in pH 5.2	×											
Green " " " 5.2		×										
Brown " " " 5.4		×										
Green " " " 5.4			×									
Brown " " " 5.6			×									
Green " " " 5.6			×									
Brown " " " 5.8			×									
Green " " " 5.8			×									
Brown " " " 6.0					×							
Green " " " 6.0					×							
Brown " " " 6.2					×							
Green " " " 6.2					×							
Brown " " " 6.4						×						
Green " " " 6.4						×						
Brown " " " 6.6						×						
Green " " " 6.6						×						
Brown " " " 6.8							×					
Green " " " 6.8							×					
Brown " " " 7.0							×					
Green " " " 7.0							×					
Brown " " " 7.2								×				
Green " " " 7.2							×					
Brown " " " 7.4									×			
Green " " " 7.4							×					
Brown " " " 7.6												×
Green " " " 7.6										×		

taken from the same culture or aquarium are placed in identical spring water cultures save for the concentration of the hydrogen ions and favorable reactions are repeatedly to be noted in the culture of low hydrogen ion concentration while unfavorable reactions are always to be noted in the culture of high hydrogen ion concentration, undoubtedly the pH of the culture must be a strong factor in determining this difference in the reactions

Histology.

The histology of reduction, dedifferentiation and resorption in Hydra has been observed by E. Shultz ('06) and W. Rehm ('25). Huxley and DeBeer ('23) also described histologically dedifferentiation and resorption in *Obelia* and *Campanularia*. Our observations are almost in exact agreement with those of the above.

Studies on reduction, dedifferentiation and resorption always
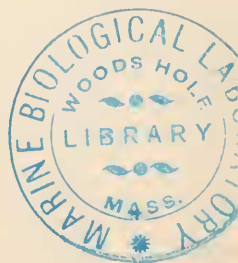
involve the histology of the animal. Hydra is a diploblastic animal having only an ectoderm and endoderm. The ectoderm presents in its vegetative condition, epithelio-muscular, interstitial, cnidoblastic and nerve cells. The endoderm, on the other hand, is made up of epithelio-muscular, glandular, interstitial and scattered or isolated nerve cells. In the ectoderm there is no great local specialization or differentiation into regions. The ectoderm, however, shows three distinct regions: (1) the oral two-thirds in which there are scattered gland cells and a general covering of epithelio-muscular cells that are heavily charged with absorbed alimentary products; (2) a basal third that has few if any gland cells and in which the epithelio-muscular cells are usually highly vacuolated, except for those at the basal disc and (3) the endoderm of the tentacles. In this third region there are no gland cells and the epithelio-muscular cells are highly vacuolated. Thus it appears that the endoderm of the highly active or moving tentacles resembles that of the relatively quiet basal third of the body proper.

Dedifferentiation and resorption have been referred to frequently above. This has been defined as a dual phenomenon which involves a local reduction of surface. We take the presence of ectodermal elements (nematocysts being the most easily recognized) within the coelenteron or endoderm as evidence that dedifferentiation and resorption have taken place.

The question now remains: How is the surface reduced locally, and how do ectodermal elements gain their entrance into the coelenteron? As this phenomenon is most often seen in the tentacles, we have studied it there. In response to adverse environmental conditions, the cells at the tips of the polyp's tentacles coalesce or become dedifferentiated. The ectoderm is apparently affected first. Here the dedifferentiated cells, preparatory to resorption, group themselves into rounded or spheroidal masses. (Fig. 1-*A*.) Nematocysts as well as numerous cell-fragments may be seen within these aggregates. Obviously there must be some change in the non-living mesoglea as well as the living endoderm before resorption of the modified ectoderm can proceed. Dedifferentiation, therefore, starts in the endoderm. These cells, apparently, break away from the walls of the tentacles and soon assume a globular form (Fig. 1, *B*).

They migrate down the lumen of the tentacle (Fig. 1, B). Now the mesoglea breaks or is resorbed (Fig. 1, C) and the endodermal elements apparently have little trouble in finding their way to the coelenteron. The cellular masses of ectoderm, spheroidal in shape and often with contained nematocysts, together with the above mentioned dedifferentiated endo-epithelial masses, may be found in the coelenteron as far down as the basal disc. Thus the surface of the tentacle is decreased. To use the language of Huxley and DeBeer ('23) in describing a similar phenomenon in *Obelia* and *Campanularia*, "The ectodermal cells may be compared with that of a rear guard, retreating yet always maintaining an unbroken front." These histological details serve as a final criterion for determining whether dedifferentiation and resorption have taken place. But with the aid of low magnification, one can see that, as resorption proceeds, the tips of the tentacles increase in diameter, and finally appear knobbed and the involved area becomes darker and darker, The endodermal cells lining the tentacles are normally highly vacuolated. These cells, however, appropriate relatively much food during the later stages of resorption.

It is certain that this dedifferentiated and resorbed tissue is used as food by the animal because nematocysts in various stages of digestion may be found in the epithelio-muscular cells in all parts of the endoderm. This confirms Kepner and Jester ('23) in their minor claim that the ingested parts were used as food; but Kepner and Jester were misled by the occasional biting off of the tentacles. Dedifferentiation and resorption are the usual reaction.

Since it was seen that both the cells of the ectoderm and the endoderm of the tentacles were almost exactly like those of the lateral walls of the basal one third of hydra, dedifferentiation and resorption was looked for in this basal region. It was found to occur in the case of the sectioned hydra recorded in culture number 5 (Fig. 2, A). No explanation is offered for dedifferentiation and resorption being found in the basal disc in this and no other case. It was noticed, however, that in this case resorption was not found in the tentacles. Resorption has not been reported before as occurring in the basal region prior to its inception in the tentacles and peristome. All other writers state that it

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starts at the tentacles and proceeds towards the base. The peristome is affected, according to them, after the tentacles have been removed. But this specimen showed dedifferentiation only in the basal region.

Green hydra reported in culture number 6 which was carried twenty-three days without food, showed histologically only slight resorption.

Rehm ('25) says that at the end of twenty one days the body of hydra subjected to inanition was reduced to a mere rounded form, which he calls, following Will and other investigators, "Reductionskörper" (§ 371). At other places he refers to these rounded hydras as presenting planula-like pictures ("planula-ähnliches Gebilde, der Reductionskörper") (§ 382). We have carried brown hydra for twenty three days within the optimum hydrogen ion concentration. This polyp showed so little dedifferentiation and resorption that they could only be detected histologically. Under low magnification the living polyp, though reduced in size, appeared to be complete and have no broken surface. The brown hydra, as recorded in culture number 8, which was sectioned after sixteen days of inanition within the optimum hydrogen ion concentration, presented, while living, no evidence of dedifferentiation and resorption under low magnification. However, the histology of this animal shows frequent nematocysts in the coelenteron hence slight dedifferentiation and resorption must have taken place during the seventeen days of inanition. Examination on this day under the dissecting microscope disclosed no difference in appearance between the remaining hydras and the one sectioned. On the twenty third day another hydra from this culture was sectioned. From the histology of this polyp, it is seen that dedifferentiation and resorption which were shown in the histological examination of the hydra sectioned on the 17th day not only has ceased but the resorbed tissue has been digested by the polyp sectioned after twenty three days of inanition within the optimum range of hydrogen ion concentration. Similar phenomena have been observed for green hydras. For a green hydra, which had suffered 14 days of inanition at optimum hydrogen ion concentration showed slight dedifferentiation and resorption; while a second green polyp, from the same culture sectioned after twenty three days of inanition at optimum hydro-

gen ion concentration, showed no evidence of dedifferentiation and resorption.

Thus it appears that during inanition at optimum hydrogen ion concentration a crisis is reached after about two weeks. During this crisis slight dedifferentiation and resorption make their appearance. The resorbed material may supply sufficient nourishment to tide the polyp, now reduced in size, through a long period before a second crisis develops and compells the dedifferentiation and resorption of more tissue.

SUMMARY.

1. The optimum range of hydrogen ion concentration for both *Hydra viridissima* and *Pelmatohydra oligactis* lies within the range pH 7.8 and 8.0.

2. Polyps allowed to develop pronounced dedifferentiation and resorption in a high hydrogen ion concentration (low pH) were induced to completely restore their lost parts when the medium was altered to be within the optimum range of pH.

3. Hydras carried within the optimum range of pH were subjected to periods of inanition as great as twenty five days without showing any external evidence of dedifferentiation and resorption at the end of this period.

4. Histological preparation of polyps, kept for long periods without food at the optimum hydrogen ion concentration, show slight evidence histologically of dedifferentiation and resorption at a critical period. This critical period appears somewhere between ten and seventeen days after inanition within the optimum range of pH. Such microscopic dedifferentiation and resorption are not progressive; for after this critical period has passed no further histological evidence of dedifferentiation and resorption has been observed.

(b) This microscopic dedifferentiation and resorption usually appear at the tips of the tentacles; but in one case we have seen it involve the basal third of the polyp and not the tentacles.

5. Hydras subjected to long periods of inanition within the optimum range of pH accept food readily. There is, therefore, no evidence of depression given by these polyps.

6. Dedifferentiation and resorption are induced rather by unfavorable hydrogen ion concentration than by inanition.

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PLATE I.

Explanation of Figures.

Fig. 1. Longitudinal section of the free end of a tentacle of *Pelmatohydra oligactis* which had been starved twenty-four hours in spring water at pH 6.8.   This shows the inception of dedifferentiation and resorption.   The mesoglea has broken down at end of tentacle.   Rounded masses of coalesced ectodermal cells are forming (*A*).   Similar rounded masses of coalesced endodermal cells are forming (*B*); at *B'* we see a mass of coalesced endodermal cells having migrated towards the lumen of the tentacle; at *C* a mass of coalesced ectodermal cells is passing through the region of the broken down mesoglea.   ×700.

Fig. 2. A longitudinal section involving a part of the basal disc of *Pelmatohydra oligactis*.   (Culture number 5.)   This specimen had been starved nine days within optimum hydrogen ion concentration.   The inception of dedifferentiation and resorption is shown at *A*; *BGC*, basal disc glands cells; *E*, endodermal cells; *L*, lateral ectodermal cells.   ×700.

PLATE I.
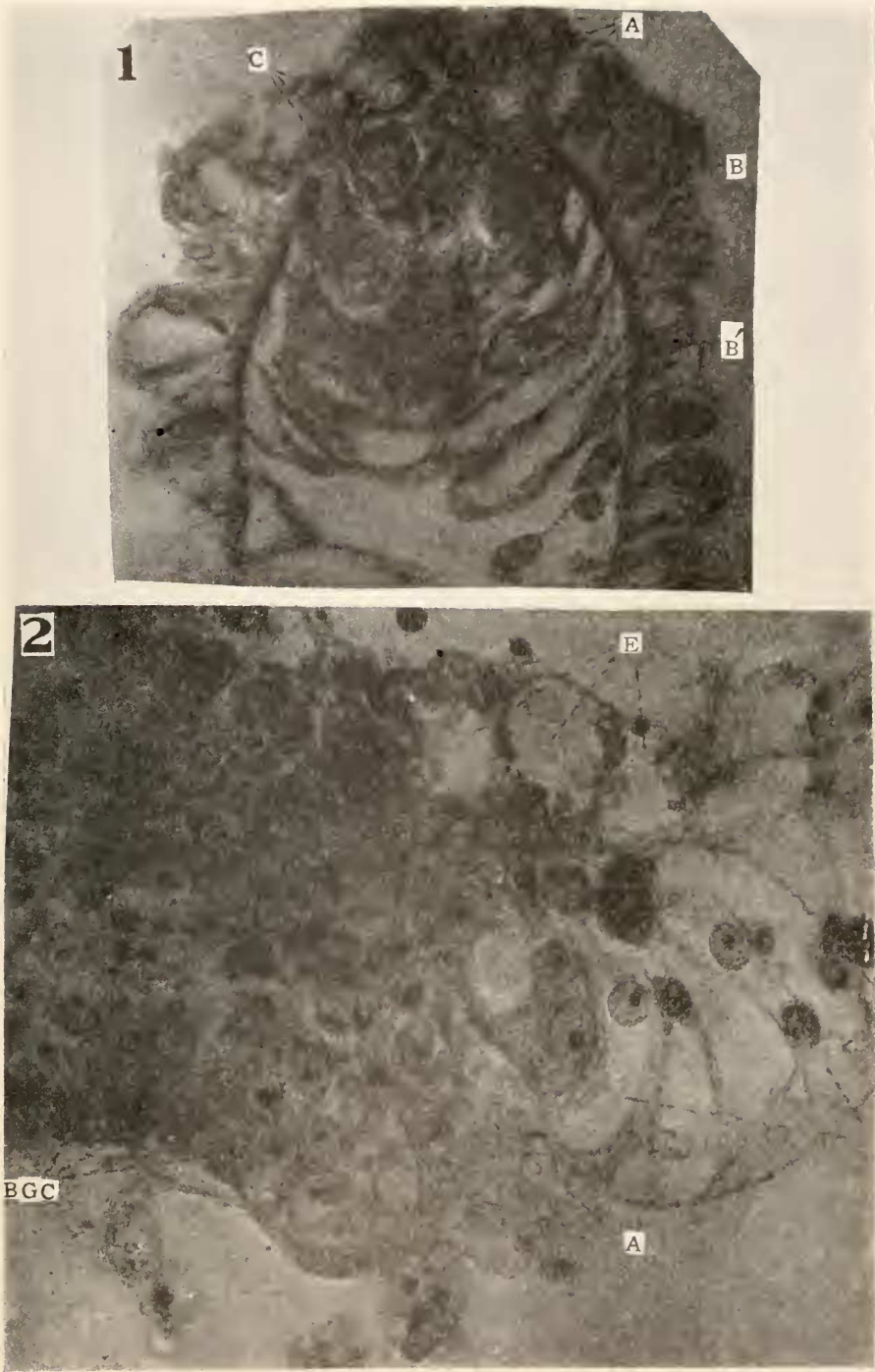
THE OCCURRENCE OF NUCLEAR VARIATIONS IN *PLEUROTRICHA LANCEOLATA* (STEIN).

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The occurrence of variations from the accepted type among the protozoa has received much attention in recent years, and a number of such cases have been reported, both of the artificially induced and spontaneously appearing sort. Most of the former have been of the "enduring modification" type, that is they persist throughout a longer or shorter period of vegetative division, but are eventually lost when conjugation or endomixis takes place. The latter may be divided into two classes. The first group would include the true mutations, of which the tetraploid *Chilodon* described by MacDougall (1925) is probably one of the best authenticated examples. In this case the mutation, which consisted in the possession of twice the usual number of chromosomes, combined with unusual size and certain other minor characteristics, persisted through both conjugation and division. To the second group would belong all other departures from normal, such as the production of monsters, the amicronucleate condition in infusoria, and various other unusual physiological and morphological characters which persist through division but tend to revert to normality eventually. Examples of this kind of variation are quite numerous. Among them may be mentioned the amicronucleate *Oxytricha* studied by Dawson (1919), the race of *Paramecium* which possessed extra contractile vacuoles (Hance, 1917), the rapidly-dividing race of *Didinium* reported by Mast (1917), and the sudden appearance of an *Arcella* having double characteristics described by Reynolds (1923). Since the latter investigator found that these abnormal characteristics could be diminished until a completely normal condition was reëstablished, or increased by selection of suitable

individuals this last variation evidently belongs with those found by Jennings (1920) and Root (1918) to exist in *Difflugia* and *Centropyxis*, with this difference, however—the former occurred suddenly, while the latter were of lesser degree and appeared more gradually. More recently Dawson (1924) has reported the occurrence of a peculiar form of *Paramecium aurelia* which has been carried in culture for several years since. The abnormal character in this case consists of a "notched" condition which is definitely heritable, at least in ordinary asexual division.

The present paper deals with variations in the number of both micro- and macronuclei in *Pleurotricha lanceolata*. Pedigreed cultures of this ciliate, which is a hypotrich belonging to the family Oxytrichidæ, were maintained for 18 months and studied mainly from the standpoint of the cytological changes occurring during conjugation and division, as described in a previous paper (Manwell, 1928).

The normal animal is shown in Fig. 1. It will be noted that it possesses two nuclei of each sort, and according to Stein (1858) who first described both the species and genus, the presence of two macro- and two micronuclei is a generic character. About two months before the culture was discontinued however, and while to all appearances it was in a very vigorous condition with division taking place very actively, individuals possessing only one macronucleus were noticed in some of the stained preparations. The micronuclear condition varied; in some cases there was only one and in others there were two as in normal individuals. Animals possessing the normal macronuclear complex but with three micronuclei have also been observed, and such changes are indeed not very uncommon, not only in *Pleurotricha* but in *Oxytricha* and other ciliates containing more than one micronucleus. But no individuals have been observed with only one macronucleus and more than two micronuclei. Fig. 2 shows an individual possessing but one nucleus of each sort in division, and in Fig. 3 a similar individual, differing only in having two micronuclei, may also be seen dividing. The next two figures show later stages in the division of such individuals, and in Fig. 6 a unimacro- and micronucleate animal is shown just after division.

From these figures it can be seen that division takes place in exactly the same way as it does in individuals having the normal

nuclear complex, and that the variations are heritable, at least in ordinary vegetative fission. To settle this point still more definitely several lines were started from individuals possessing but one nucleus of each sort and followed for 10 days. At the end of that time these subcultures were lost by accident and other circumstances made it necessary to conclude the experiment, but stained preparations made from each generation showed clearly that the reduced number of nuclei was being passed from one generation to the next.

A careful examination of stained preparations has been made in an effort to discover whether the abnormal nuclear complex was accompanied by any other morphological changes, but apparently there were none. During the early stages of division however (about the stage shown in Fig. 2) it was frequently possible to distinguish animals possessing but one macronucleus from normal individuals in the same culture in a similar stage, for the bodies of the former were definitely broader about 1/3 of the way back from the anterior end and then tended to become narrower, while in the normal animals the entire middle third of the body was of a fairly uniform width. If there were any differences in size they were in favor of those individuals possessing but one nucleus of each sort.

No evidences of conjugation among these abnormal individuals was ever observed, but since as previously reported, conjugation occurred but rarely in all the cultures from start to finish of the experiment, not much stress can be laid on this point. Encystment was also not observed. Consequently it cannot be said whether such a variation as this would survive endomixis and conjugation, although it seems probable that in some cases at least, unimicro- and macronucleate conjugants might produce similar individuals.

In view of the work of Baitsell (1914), and the fact that conjugation in this species has been shown to result, at least when it occurs under cultural conditions favorable to vegetative division, in almost 100 per cent. mortality (Manwell, 1928) the question of the occurrence of such morphological variations as herein described becomes of some practical importance. For obviously, if under favorable conditions multiplication by fission can continue indefinitely, then such changes might be perpetuated for a very

long time in nature, as well as in artificial cultures. And if this is so account should be taken of the fact in the description of genus and species, since the number of nuclei, especially of the macronuclei, is a conspicuous character. If asexual reproduction can continue indefinitely then the sudden appearance of changes of the kind described would, for practical purposes, have the value of a mutation.

The occurrence of abnormal micronuclear conditions has been reported a number of times before, particularly with respect to the total absence of a micronucleus, and the presence of one or two supernumerary micronuclei is not very uncommon in species ordinarily possessing two or more, as already noted, but apparently the number of macronuclei is a much more constant character. The only instance in which a variation in the latter has been reported, to the author's knowledge, at least, is that given by Calkins (1926). Here he states (p. 579) that in early cultures of *Uroleptus mobilis* the number of macronuclei was almost uniformly 8, but as the age of the cultures increased individuals with a greater number of nuclei became common, until finally the number was nearly always 14 or 15.

Summary and Conclusions.

In a pedigreed culture of *Pleurotricha lanceolata*, a species o, hypotrich normally possessing two macro- and two micronuclei individuals with only one macronucleus and one or two micronuclei suddenly appeared, at a time when division was rapid and the culture apparently very vigorous.

That the difference in nuclear number was heritable, at least in asexual multiplication, was shown from stained preparations and pedigreed lines, and the fact that it has been shown that this species will live and divide normally apparently indefinitely under favorable conditions, without conjugation, makes it probable that such variations as have been described would continue for a very long time, and that animals with such peculiarities may be common in nature as distinct varieties.

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EXPLANATION OF THE FIGURES.

Magnification × 550; all drawings made with camera lucida

PLATE I.

FIG. 1.   A typical vegetative individual.

FIG. 2.   An individual with one macronucleus and one micronucleus in a moderately early stage of division.

FIG. 3.   A division stage similar to the above in an animal having two micronuclei, but only one macronucleus.

FIG. 4.   A more advanced stage in an individual similar to the above.

FIG. 5.   The final stage of division in a unimicro- and macro-nucleate individual.

FIG. 6.   A daughter individual just after fission.
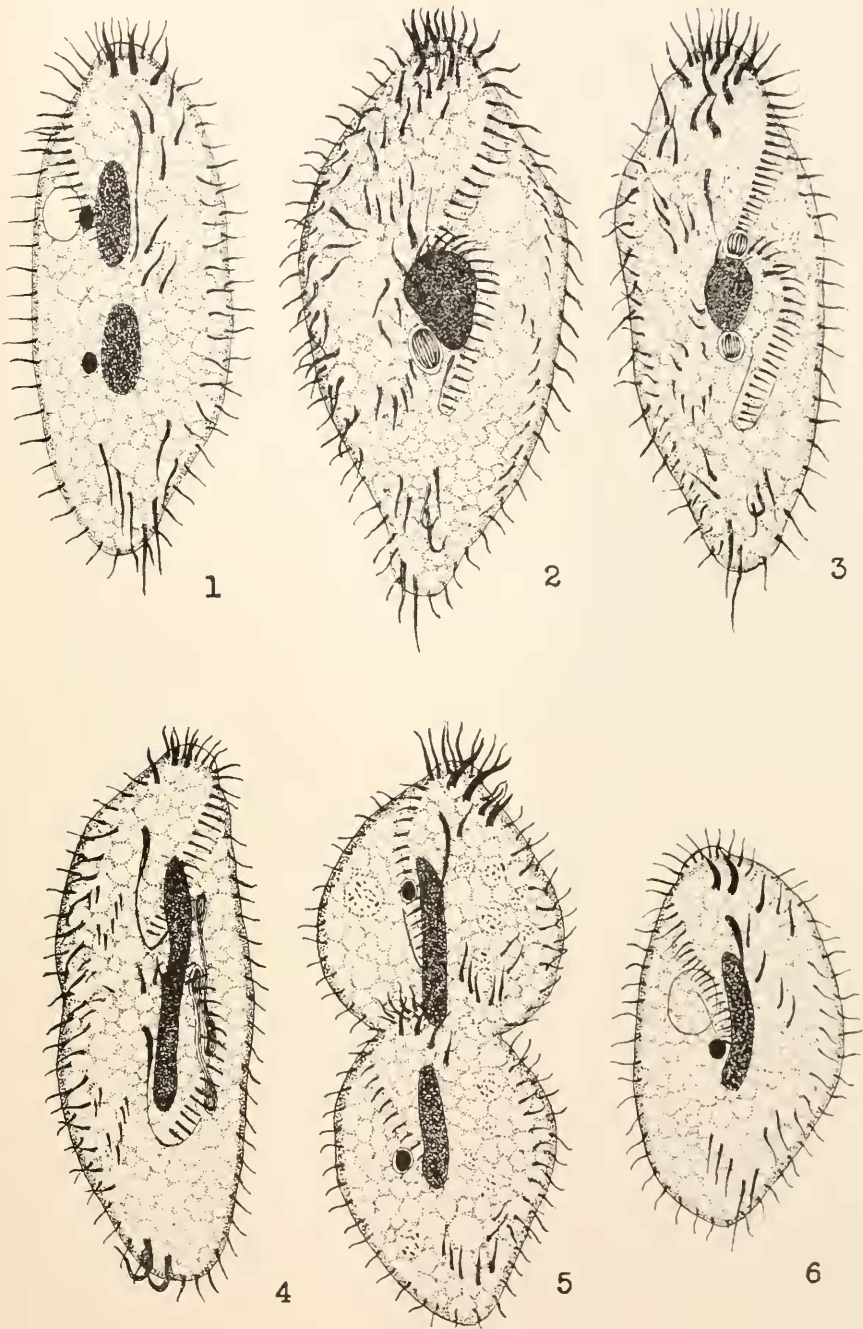
OBSERVATIONS ON THE LIFE HISTORY AND PHYSIOLOGICAL CONDITION OF THE PACIFIC DOG FISH (*SQUALUS SUCKLII*).

J. P. QUIGLEY.[1]

Incidental to an investigation of the reactions of *Squalus sucklii* to variations in the salinity of the surrounding medium (1) observations were made regarding the life history and physiological condition of this fish.

The fish were captured during the months of June, July and August of 1926 from the Straits of Georgia in the vicinity of Departure Bay, Vancouver Island, B. C. They were taken on a set line, the hooks of which were baited with pieces of salted herring. Most of the fish were obtained at a depth of about 30 meters, and they were generally caught near kelp beds. A sample of water taken at a depth of 30 meters in the region where many of the fish were taken was found by Lucas (2) to have the following characteristics; pH 8.4, temperature 10.3° C., density 1.0218, oxygen content 4.41 cc. per liter, sodium chloride content 27.37 gm. per liter.

Weight of Fish.—It was found that many of the factors associated with the weight of the fish could be emphasized by grouping the fish according to weight as has been done in Table I. Examination of this table shows that with the fish of lighter weight the two sexes are nearly equally represented, the number of males being slightly greater. As heavier fish are considered, the relative number of males shows a marked increase, then a sudden decrease so that in the weight divisions above 4,000 grams the males are entirely absent.

These results probably indicate that male fish with body weight over 4,000 grams do not exist in this locality during the summer. It cannot be definitely stated that the figures obtained with fish of lighter weight indicate the relative proportion in which the

TABLE I.

Weight Limits (Grams).	Number of Fish Obtained.	Number of		Percentage.		Average Length (Cm.).	Average Increase in Length.
		Males.	Females.	Males.	Females.		
300–399...	12	7	5	58	42	39.9	
400–499...	15	9	6	60	40	43.6	3.8
500–599...	16	11	5	69	31	45.7	2.1
600–699...	13	8	5	62	38	48.4	2.7
700–799...	5	5	0	100	0	52.5	4.1
800–899...	7	4	3	57	43	53.8	1.3
900–999...	5	5	0	100	0	54.7	0.9
1,000–1,499 .	22	20	2	91	9	60.3	5.6
1,500–1,999 .	11	9	2	82	18	69.2	8.9
2,000–2,999 .	30	26	4	87	13	74.9	5.7
3,000–3,999 .	13	5	8	38	62	83.3	8.4
4,000–4,999 .	16	0	16	0	100	90.5	6.2
5,000–5,999 .	16	0	16	0	100	91.6	1.1
6,000–6,999 .	4	0	4	0	100	95.5	3.9
7,000–7,999 .	1	0	1	0	100	99.0	3.5

two sexes occur, although such probably is the case. Since the fish were taken on a set line hunger or greed might conceivably be a factor in determining whether or not fish would take the bait. The stomach of fish captured usually contained much food, a fact which indicates that feeding for this fish is determined more by the availability of food than by hunger.

Out of 219 fish captured, 128 (58 per cent.) were males. Craigie (3) examined the fish obtained in the same region during July and August, 1925, and found that among 76 specimens 44 (60 per cent.) were males, while during December of 1925 by examining 117 specimens he found 47 (40 per cent.) males.

As was to have been expected, there is a comparatively definite relationship between weight and length of fish. The increase in length is rather steady though not entirely uniform as heavier fish are compared with those of lighter weight. It could not be shown that sex altered the relation of weight and length. There was a slight though inconstant indication that nonpregnant females were longer than pregnant females of the same weight. The longest fish captured measured 99 cm., the shortest 35.5. The heaviest fish weighed 7,550 grams and the lightest 300 grams. When increasing their weight 100 grams the smaller fish made an increase in length of approximately the same magnitude as did the larger fish when making a weight increase of 1,000 grams.

Pregnancy and Embryos.—Of the females captured, 43 per cent. carried embryos large enough to be readily noted in a cursory inspection. The lightest fish having embryos weighed 3,440 grams and was 85 cm. in length. These figures give an approximate minimum limit of the size of the mature female. Among the 50 females captured with a weight equal to or above 3,440 grams, 39 (78 per cent.) carried embryos.

Ford (4) quotes the conclusion of several investigators that *Squalus acanthias* breeds throughout the year and of other investigators that this species breeds only during certain periods. The results of his own investigations support the latter conclusion and tend to show that near Plymouth, England, specimens ready for birth would not be found earlier than the end of August. I found specimens of *Squalus sucklii* embryos at all times during the summer which ranged through all the sizes from the smallest to those with the umbilical scar healed completely and apparently ready for birth. This observation naturally suggests that in the vicinity of Nanaimo, *Squalus sucklii* breeds at all times of the year.

In any one parent, the embryos were of the same general size. A set of developing eggs was always found in females carrying embryos. The number of embryos obtained from 16 fish varied between 3 and 11 with an average number of 6.87. Although it could not be definitely stated that none of the embryos had been lost from the mother in the course of capture it is believed that this was a rare occurrence. No embryos were lost after the mother was taken from the set line and in most cases egg capsules still unruptured were obtained. In an examination of Squalus acanthias Ford (4) found that females of this species could carry as many as 11 embryos but the greatest number of pregnant fish carried only 3. In *Squalus sucklii* I found that embryos of both sexes usually occurred in the same uterus but there was no relation between the number of either sex, *e.g.* in one fish I found 6 females and 1 male, in another 3 males and no females. Of the embryos obtained 50 per cent. were males. This figure is to be contrasted with that previously noted for the fish of small size taken on the set line where a preponderence of males existed. A blue shark, *Prionace glance*, (identified by Professor J. R. Dymond) received at the Pacific Biological Station, August 19,

1926, was found to have 11 females and 8 male embryos all the same size nearly ready for birth.

Constitution of Shoals.—Throughout the period fish were being taken, the specimens obtained on any set line usually consisted of both sexes in approximately equal numbers and of all sizes. The conclusion was reached that the shoals consisted of both sexes and all sizes of fish or else the line had been visited within a few hours by several different shoals. It was also noted that the largest fish were usually taken at a greater depth (very near or actually on the sea bottom) than the smallest and it may be that the composition of shoals is in part determined by size. From his study of *Squalus acanthias*, Ford (4) concluded that for this species the mature males and females each form separate shoals while these shoals in turn are distinct from those composed of immature males and females together. I obtained fish in the same region throughout the summer. It is therefore likely that certain shoals inhabit this region during the entire season.

SUMMARY.

1. Among the smaller fish males were slightly more prevalent than females. Males weighing more than 4,000 grams were not obtained. Females attain a much greater length and weight than males. The greater weight of the females was not always due to the presence of eggs or embryos.

2. A comparatively definite relationship exists between weight and length of fish. The relationship of length increase to weight increase for small fish is approximately ten times as great as for large specimens.

3. Of the mature females captured 78 per cent. carried embryos. This species apparently breeds throughout the year. The average number of embryos carried by the females is greater than six.

4. The shoals apparently consist of fish of all sizes and of both sexes. The shoals probably remain in the same region throughout the summer.

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ALGÆ OF PONDS AS DETERMINED BY AN EXAMINATION OF THE INTESTINAL CONTENTS OF TADPOLES.

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INTRODUCTION.

During the last few years a considerable amount of research has centered around the food taking of small fresh-water fish. This work has emphasized the dependence of small fish on algæ and in turn these fish as a source of food for the game fish. In reviewing literature the writer has found comparatively little scientific work on the feeding habits of the tadpole and frog.

The tadpole as well as the small fish is an indirect source of food for the human race. Tiffany ('22) states: "For most of the young fishes examined the complete story reads: 'no phytoplankton, no gizzard shad.'" It may also be said, no algæ, no tadpole.

The writer wishes to express her gratitude to Dr. Bruce D. Reynolds, who suggested this problem and who has greatly assisted by his advice and criticism in the preparation of this paper; also to Professor I. F. Lewis and Dr. E. M. Betts for helpful criticisms.

METHODS.

During the summers of 1927 and 1928 one hundred tadpoles and one hundred pond collections were taken from five ponds on the campus of the University of Virginia and in the surrounding vicinity. Two of the ponds measured approximately 250 ft. x 100 ft., one 150 ft. x 50 ft., one 100 ft. x 30 ft., and one 50 ft. x 20 ft. The ponds which were studied did not have active outlets.

Two examinations of each of these ponds were made during the summer of 1927 from July 15 to August 28, and two were made during the summer of 1928 from June 20 to July 5. Each collection from a pond consisted of five tadpoles [1] which measured from

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[1] Of the 100 tadpoles used in these experiments, 94 were *Rana clamitans* and 6 *R. catesbeiana*.

one and three-fourths inches to five inches long and five collections of sediment taken from the edges of the ponds. The tadpoles and pond collections were put in separate containers. Immediately after returning to the laboratory the tadpoles were killed and the intestines removed. Three slides were made of material taken from each digestive tract, one from the anterior and one from the middle regions of the small intestine, the third from the anterior region of the large intestine. A study of each of the slides was made under the high power of the microscope. The algæ from each region were identified and recorded. The pond collections were studied in a similar way. Three slides were made from each of the pond collections. The algæ from each slide were identified and recorded.

During the summer of '27 the tadpoles were collected from the pond, and then the pond collections were made without any effort to correlate the position of the tadpole and the pond collection, but in the collections made during the summer of '28 a tadpole was caught and from the same place a pond collection was made.

THE PROBLEM.

The experiments presented in this paper were not undertaken primarily for the purpose of studying the food of tadpoles, but rather in order to ascertain if the algæ found in the alimentary tract of tadpoles can be relied upon as an index to the microscopic flora of the ponds in which the tadpoles are living. In other words, does the tadpole feed on different kinds of algæ or is it selective in its feeding habits? If not selective, is it as good a collector of algæ as the investigator interested in studying them?

EXPERIMENTAL.

In following up this problem observations were made on four collections, made at different times, from each of five ponds. The results obtained are shown in tabular form.

By referring to Table I. it will be seen that the number of species of algæ obtained from the intestine of the tadpoles exceeded the number obtained from the pond collections in every case except two, and in these instances they were the same—the pond collections being made where the tadpoles were caught.

. Attention is also called to the relative number of algæ found in

the intestines of tadpoles and the ponds from which they were taken, in large and small ponds (Table I.). It is evident that, when making collections from small ponds, the investigator is able to find most of the algæ present; whereas if the pond is a large one there is an appreciable difference between the number of species of algæ obtained by the two methods—the ratio being approximately 4 : 3 in favor of the tadpole.

TABLE I.

SHOWING THE TOTAL NUMBER OF SPECIES OF ALGAE TAKEN FROM THE INTESTINAL TRACT OF FIVE TADPOLES AS COMPARED WITH THE TOTAL NUMBER FOUND IN FIVE COLLECTIONS MADE FROM THE SAME PONDS.

Size of Pond.	Collections Made during Summer of 1927.				Collections Made during Summer of 1928.			
	Jun. 15–Aug. 11.		Aug. 11–Aug. 28.		Jun. 21–Jun. 27.		Jun. 27–July 5.	
	Tadpole.	Pond.	Tadpole.	Pond.	Tadpole.	Pond.	Tadpole.	Pond.
250 x 100 ft......	50	32	59	39	63	49	58	48
250 x 100 ft......	54	42	45	37	44	44	56	49
150 x 50 ft......	52	46	47	44	65	56	59	46
100 x 50 ft......	35	30	63	50	56	47	47	41
50 x 20 ft......	35	30	46	44	47	39	44	44

As stated in a paragraph under Methods, three examinations were made of each pond collection and of each tadpole—one from the anterior region of the small intestine, one from the middle region of the small intestine, and one from the large intestine. Table II. shows the distribution of the species in different regions of the intestinal tract as compared with the total number found in the tadpole and the total number found in the pond collections. Usually more species of algæ were found in the anterior end of the small intestine, but there is not a great variation in numbers in the three regions. Most of the algæ found in the large intestine show slight evidence of having been acted upon by the digestive juices.

Even though the species of algæ found in the tadpoles outnumbered those in the pond collections, algæ which did not occur in the tadpoles' intestines were found in collections made from the pond. There was one exception, and in this case the tadpole and pond collection were taken from the same place. In this entire work only five species of algæ were found in pond collections

TABLE II.

SHOWING THE TOTAL NUMBER OF SPECIES OF ALGÆ FOUND IN DIFFERENT PONDS, THE NUMBER FOUND IN TADPOLES AND THE NUMBER FOUND IN DIFFERENT REGIONS OF THE INTESTINE.

A. S. Int., anterior end of small intestine; M. S. Int., middle region of small intestine; A. L. Int., anterior end of large intestine.

Pond.	Tadpole.	A. S. Int.	M. S. Int.	A. L. Int.
29	36	23	22	20
30	34	22	16	19
35	44	23	24	19
33	33	24	19	19
30	45	21	24	31
28	33	28	26	21
31	40	26	20	24
32	31	19	14	14
27	50	26	21	36
16	34	18	13	16
25	35	29	12	16
18	35	26	14	22
35	34	21	26	16
24	36	21	21	27
32	38	21	16	21
27	36	20	22	22
32	34	31	15	18
25	38	22	21	25
24	32	18	18	19
36	49	32	26	30

TABLE III.

COLLECTIONS MADE DURING SUMMER OF 1927.

Total Number Species from Both Sources.	Percentage of Those Found in Tadpoles.	Percentage of Those Found in Pond.	Total Number Species from Both Sources.	Percentage of Those Found in Tadpoles.	Percentage of Those Found in Pond.
50	86.20	55.17	70	82.85	55.71
68	79.32	61.76	58	83.10	63.79
70	74.28	65.71	57	82.62	77.19
45	77.77	66.66	68	93.64	73.23
37	94.59	81.08	53	86.79	75.28

COLLECTIONS MADE DURING SUMMER OF 1928.

67	94.03	73.13	64	95.31	77.50
50	88.	88.	58	96.55	84.48
66	98.48	84.84	62	95.17	74.19
60	94.33	78.33	56	100.	83.91
48	97.91	81.25	47	93.61	93.61

Showing total number of species of algæ taken from each pond, including the percentage of those obtained from tadpoles and from pond collections.

which were not also observed in the tadpoles. Evidently these species were very rare, for only one was encountered the second time. The fact that these algæ were not found in the tadpoles does not indicate, therefore, that the tadpoles refuse to eat them.

The variation in percentage of algæ from the two sources is less when pond collections and tadpoles are taken from the same place. This may be seen by referring to Table III. The pond collections made during the summer of 1928 were taken from the immediate vicinity in which the tadpoles were caught, while those made during the summer of 1927 were taken without regard to this matter.

SUMMARY.

It is a well known fact that tadpoles feed on microscopic plants. The importance of this animal as a collector of algæ is clearly demonstrated. In comparing the intestinal contents of one hundred tadpoles with pond collections made from the same ponds, the number of species of algæ obtained from the tadpoles exceeded the number obtained from the collections in every case except two; and in these instances, they were the same. It may be stated, therefore, that an examination of the intestinal contents of tadpoles affords one of the best and easiest methods of determining the species of algæ present in ponds. This is especially true in large ponds, and applies particularly to the phytoplankton.

In this examination one hundred and seventy species and varieties of phytoplankton were found. Of this number, one hundred and sixty-five were encountered in the intestines of tadpoles.

CONCLUSION.

1. The food of green-frog tadpoles consists chiefly of algæ.

2. The algæ from pond collections and from the intestinal contents of tadpoles taken from the same ponds do not differ as much in small ponds as they do in the larger ones.

3. The anterior region of the small intestine is considered to be the best region for making examinations for algæ.

4. The species of algæ taken from the intestines of tadpoles constituted, on the average, 89.73 + per cent. of the total found.

5. An examination of the intestinal contents of tadpoles affords one of the best and easiest methods of obtaining a collection of algæ from ponds.

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FURTHER OBSERVATIONS ON THE CHEMICAL COMPOSITION OF WOODS HOLE SEA WATER—THE CHLORINE CONTENT AND SALT ANALYSIS.

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From time to time we have had occasion to make further observations on the sea water at Woods Hole since the publication of the original analysis (1). Though not in any sense complete it is believed that the following data may prove useful and therefore they are presented.

It should be pointed out that our aim has been always to select methods of analysis which would adapt themselves to the use of relatively small fluid volumes, as only in this way can they become applicable to the investigation of physiological and biological problems. From the large number of analyses of sea water tabulated by the Hydrographic Laboratory of Copenhagen, Knudsen, Dittmar (2) etc., further data of this kind have oceanographic interest but little more. There has, therefore, been made a conscious attempt to utilize more sensitive methods which require small samples for analysis, albeit the absolute values *may* not be quite as accurate.

DETERMINATION OF CHLORINE.

Since many physiological activities are sensitive to slight changes in the tonicity of the surrounding medium it seemed of interest to determine whether the chlorine content of the Woods Hole sea water varied to a significant degree from day to day. The method employed was as follows: Standard $AgNO_3$ was made such that 1 cc. was equivalent to 10 mg. chlorine. This was standardized against pure NaCl since it has been shown by Thompson (3) that this salt may be substituted for standard water from the Hydrographic Laboratory. The $AgNO_3$ was

kept in the dark in a glass stoppered brown bottle and the standardization repeated at the end of the series of determinations. The method, thereafter, followed in detail that presented by the Association of Official Agricultural Chemists (4). The burette used was of 50 cc. capacity, standardized by the Bureau of Standards, Washington. 15 cc. samples of sea water were measured with a standardized pipette and diluted with distilled water to 35 cc. before titration.

Samples were taken from the laboratory tank. This tank is fed by water taken about 125 feet from shore. The other samples were taken from surface water as follows: (1) Buzzards Bay one half mile North of Robinson's Hole. (2) Cuttyhunk 300 feet from shore on the "Sound" side. (3) Tarpaulin cove one half mile out in the Sound; water 80 feet deep. (4) East of Nobska; water 28 feet deep.

Duplicate titrations were made and it may be said that these determinations but rarely disagreed.

The temperature was taken with not great accuracy, employing a standard 50 degree laboratory thermometer. Such slight changes as observed during these observations were not considered significant.

Grams of chlorine per kilogram were calculated from Thompson's empirical formula—

$$Cl_w = 0.008 + 0.99980\ Cl_v - 0.001228\ Cl_v^2$$

where Cl_w = grams of Cl per kilogram and Cl_v = grams Cl per liter at 20° C. A graph prepared by using the more common range of Cl contents was found useful.

The salinity—defined as the weight in grams of all the salts dissolved in a kilogram of sea water, after the carbonates have been converted to oxides, the Br and I have been replaced by Cl and the organic matter has been completely oxidized—was calculated from the relation derived by Knudson—

$$So/oo = 0.030 + 1.8050\ Cl_w$$

Of course it must be recognized that this is only an approximation, as Giral (5) has emphasized.

During these observations it should be stated that the weather was in general extremely bad, rain alternating with fog for dis-

TABLE I.

CHLORINE CONTENT OF WOODS HOLE SEA WATER DURING THE SUMMER OF 1928.

Date.	Source.	Temperature.	Grams Cl per Liter.	Grams Cl per Kilogram.	So/oo.
July 16...	Laboratory tank	21 degrees	17.80	17.42	31.47
" 18...	" "	22 "	17.80	17.42	31.47
" 21...	" "	21 "	17.86	17.48	31.58
" 23...	" "	21 "	17.77	17.39	31.42
" 26...	" "	21.8 "	17.77	17.39	31.42
" 28...	" "	21 "	17.86	17.48	31.58
August 1 .	" "	20.5 "	17.80	17.42	31.47
July 17...	Buzzards Bay..	20 "	17.93	17.54	31.69
" 17...	Cuttyhunk	20 "	18.00	17.60	31.79
" 21...	Off Tarpaulin Cove	20 "	17.93	17.54	31.69
" 21...	East Nobska	20 "	17.70	17.32	31.27

agreeably long intervals. The results, do not show any very marked changes in the Cl content of the water but it is altogether possible that a dry summer may increase the Cl content. Samples taken from other points along the uneven coast of Woods Hole show more evident variations, as was to be expected.

SEA SALT ANALYSIS.

Samples of the dried sea salt taken from the laboratory tank during the summer of 1926 have been analysed, employing the classical methods as given in the Bulletin of the Official Agricultural Chemists (4) and by Scott (6). Though not complete, these data are presented, as they may be found useful.

SEA SALT OF WOODS HOLE.

	Percentage.	
	No. 1	No. 2.
Sodium	30.68	30.49
Magnesium	3.31	3.48
Calcium	1.27	1.12
Silica	0.014	0.018
Phosphate	Trace	Trace
Nitrate	Trace	Trace

The above analyses would tend to confirm the suggestion made in our former paper that the Kramer-Gittleman direct method for the determination of sodium, while very convenient for relative data, may give an absolute value which is low. One must remember, however, that using the Haywood and Smith Method

(7) or that of Dittmar the sodium determination comes out low, as has been the universal experience of analysts. The values are then corrected by employing Dittmar's method (2) of "total sulphates." The older methods for sodium determinations are so cumbersome (as reference to Dittmar's article will show) that there is still some doubt as to the accuracy of the results.

During the Summer of 1928 we have again confirmed Atkins' (8) and Harvey's (9) work on the nitrates and phosphates. Samples of the Woods Hole water showed only the smallest trace of NO_3 and PO_4 during July 1928, the time at which our analyses were made this year. This change is, as they have shown, due to seasonal variations in the plankton.

SUMMARY.

1. The chlorine content of Woods Hole sea water has been examined over a three-week period and shown not to vary within any large range.

2. Analyses of the sea salt are presented.

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THE PRECIPITATION OF CALCIUM AND MAGNESIUM FROM SEA WATER BY SODIUM HYDROXIDE.

ELEANOR M. KAPP.[1]

In the course of an investigation into the modification of sea water for use as a perfusion medium (Kapp, '28), it became necessary to know something of the relative amounts of calcium and magnesium precipitated by sodium hydroxide. Haas ('16) suggested that the first flat portion of his titration curve for sea water was coincident with the precipitation of Mg as hydroxide, the second with that of Ca. That this was a reasonable assumption is further suggested by the solubility product constants for the hydroxides of Mg and Ca, which are 1.2×10^{-11} and 4.1×10^{-6}, respectively (Johnston, '15). To obtain more exact information concerning this behavior of Mg and Ca, the following experiments were run on sea water taken from the English Channel outside the Plymouth breakwater, and from Great Harbor, Woods Hole, Mass.

Graded amounts of 10 normal NaOH (practically carbonate-free [2]) were added to 100 c.c portions of sea water. The flasks were stoppered and the contents thoroughly mixed. The supernatant fluid was filtered off as soon as the precipitate had settled somewhat (within four hours in all cases), and Ca and Mg were determined in separate samples of the filtrate. Ca was precipitated as oxalate from 25 cc. samples according to McCrudden's ('09) method, and allowed to stand in the refrigerator for at least 18 hours. The oxalate, after washing, was determined with permanganate. The Mg determinations were carried out according to the method of Willstätter and Waldschmidt-Leitz ('23) on duplicate 5 cc. samples from each filtrate. Values for total Ca and Mg were obtained by the same techniques from samples of untreated sea water, and show good agreement with the figures compiled by Clarke ('24) for sea water from a wide range of sources.

[1] From the Laboratory of the Marine Biological Association, Plymouth.

[2] Made up from the filtrate of a 50 per cent. solution in which the carbonate had been allowed to settle.

The behavior of Mg and Ca was investigated by Irving [1] ('26), but major emphasis was placed by him on equilibria within the biological limits of alkalinity. An extension of these investigations and an explanation of certain discrepancies which were encountered follow.

The data for Mg for Plymouth sea water are given in Fig. 1, and roughly agree with my results obtained on Woods Hole sea water by a less reliable technique. The curve for the precipitation of Mg as drawn by Irving is inaccurate, as owing to the scarcity of his points he completely missed the plateau. Fig. 1, however, substantiates the points he did determine.
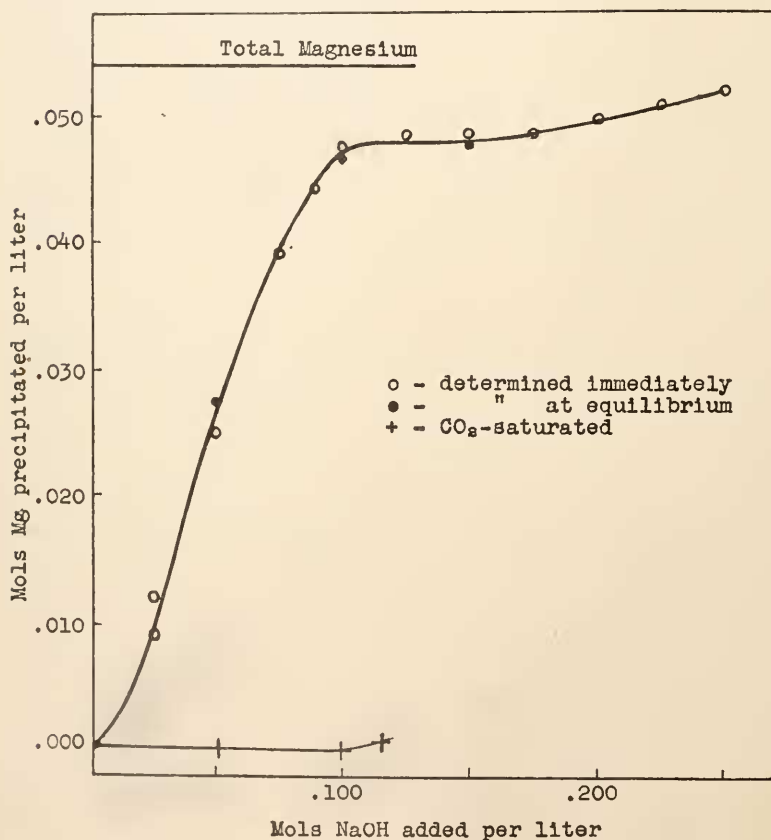
FIG. 1. The precipitation of Mg from Plymouth sea water in relation to the amount of NaOH added.

[1] Unknown to me when this work was undertaken.

The data for Ca show that the results may be considerably modified by a slight variation in procedure. The Ca curves plotted as hollow circles in Figs. 2 and 3 both differ markedly from the one obtained by Irving. His technique was substantially the same as mine, with the exception that his original samples of sea water, after the NaOH had been added, were shaken for 24 hours instead of being filtered at once, so that equilibrium was insured. Since $CaCO_3$ tends to remain supersaturated, it was suspected of being the cause of the discrepancy. A control experiment was therefore set up, in which the NaOH was added very slowly as a normal (instead of 10 normal) solution, in order to avoid local high concentrations of hydroxide, and the stoppered mixtures were allowed to stand with occasional shaking for one week. At the end of this time they were filtered and analyzed.
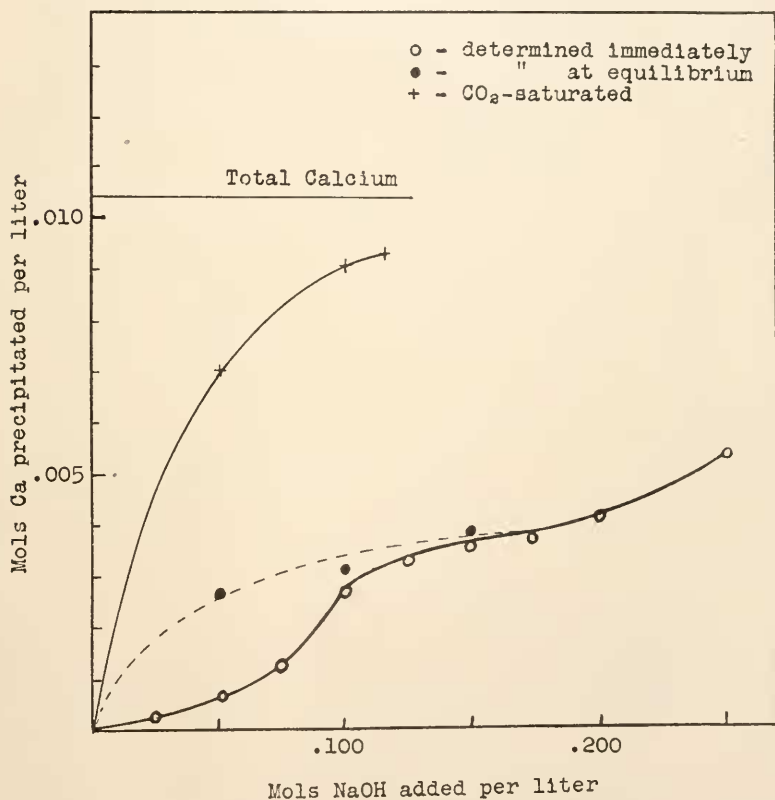
FIG. 2. The precipitation of Ca from Plymouth sea water in relation to the amount of NaOH added.

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The Ca curve thus obtained differs from the first ones, this time confirming the results of Irving. Its points are shown in Fig. 2 as black circles. The difference between the two curves is therefore due only to the slowness with which $CaCO_3$ is precipitated, and can be controlled by taking the time factor into account. The same situation does not exist in the case of Mg, as can be seen from the black circles plotted in Fig. 1, which coincide with the original curve.

The effect of increasing the amount of carbonate was obtained by saturating several samples of sea water with CO_2 before the addition of the alkali. Increasing quantities of normal NaOH were then added very slowly, to allow the gelatinous precipitate which formed to redissolve, until the third sample, to which 11.5 cc. had been added, remained cloudy. The mixtures were aërated to drive off excess CO_2, and allowed to stand in contact with the atmosphere for one week. During this time a crystalline precipi-

Total Calcium

Mols Ca precipitated per liter

.010

.005

.100   .200

Mols NaOH added per liter
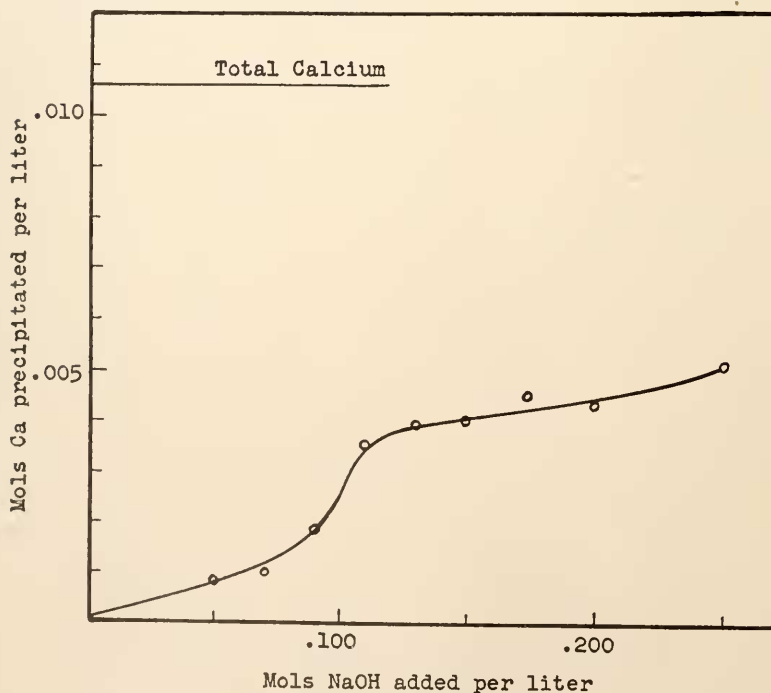
FIG. 3. The precipitation of Ca from Woods Hole sea water in relation to the amount of NaOH added.

tate had formed, and the solutions were filtered and analyzed as before. The results are shown by the crosses in Figs. 1 and 2, and are strikingly different from the other precipitations. In this case the addition of a small amount of alkali precipitates only the Ca, while the Mg is affected by larger amounts.
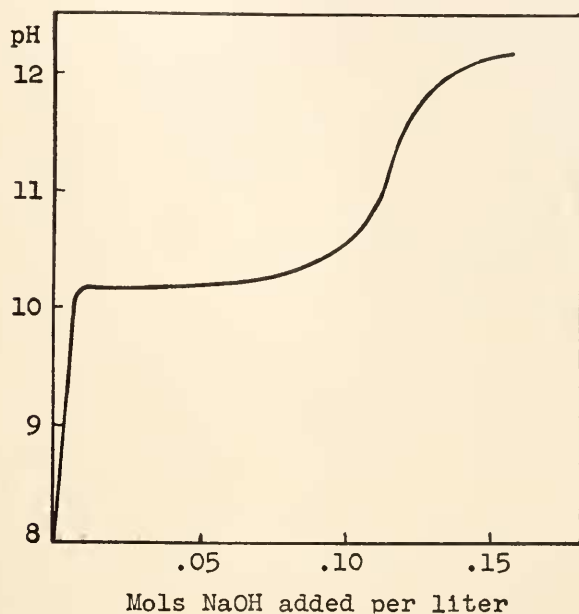
FIG. 4.   The effect of NaOH on the pH of sea water (after Haas).

With reference to the reason for the shape of the Haas titration curve (Fig. 4), it is clear that Haas' own statement, mentioned previously, must be modified somewhat. As he suggested, Mg is precipitated rapidly by NaOH over the range where his titration curve shows a plateau. At a region corresponding to the addition of 0.1 mols of NaOH per liter of sea water, the titration curve begins its second rise, and the Mg curve flattens out. A small amount of Ca, however, is precipitated throughout, owing its first precipitation to the insolubility of the carbonate,[1] which is intermediate in this respect between Mg and Ca hydroxides.

I am deeply indebted to Dr. E. J. Allen, F. R. S., of the Marine Biological Association, Plymouth, for facilities extended to me

[1] $K_{S.P.} = .98 \times 10^{-8}$ (Johnston, '15).

during this investigation. I also wish to thank Prof. M. H. Jacobs and Mr. H. W. Harvey for their helpful interest.

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FURTHER OBSERVATIONS ON THE EFFECT OF HIGH FREQUENCY SOUND WAVES ON LIVING MATTER.

E. NEWTON HARVEY, ETHEL BROWNE HARVEY AND
ALFRED L. LOOMIS.[1]

Interest in the biological effects of very high frequency sound waves started with the investigations of Wood and Loomis (1) who devised methods for producing intense "supersonic" vibrations and described many of the phenomena connected with them. The reader is referred to this paper for a description of the two kilowatt generator and methods of working with the waves. The apparatus was of such high power and the sound waves of such great intensity as to produce considerable heating. It seemed highly desirable in working with cells to reduce the heating effects of the vibrations, and to observe the cell with the microscope while being radiated. After many attempts to use the high power oscillator as the source of the waves and to lead them to the material on the stage of a microscope along capillary rods and tubes, a low-powered apparatus was decided upon as the most convenient for the purpose. This has previously been described by Harvey and Loomis (2) together with some of the effects of these supersonic waves on living organisms, cells and tissues. The outfit consists of a 75 watt high frequency oscillator and a quartz crystal whose vibrations, produced in the electric field by reversal of the piezo-electric effect, travel through any medium in contact with the crystal. A frequency of 400,000 per second was used and the material mounted directly on the crystal which served as a microscopic slide. Schmitt, Olson and Johnson (3) have also described various biological effects using a 250 watt generator with crystal immersed in xylene. They lead the sound waves along a rod of small diameter ending in a microneedle, which could be inserted into the material to be studied.

Some additional effects have been recently observed with our

75 watt outfit in its original form and also modified to use higher frequencies by changing the capacity, inductance and crystal. The new quartz crystal was a spectacle lens which happened to be cut in the proper direction, kindly loaned by Dr. Kenneth Cole. The natural frequency of this crystal was approximately one and one quarter million per second. Its thickness varied from 1 to 1.8 mm. and consequently the distance between the tin foil electrodes, was much less than in the original 7 mm. crystal, giving a far more intense electrical field and greater effects. A few experiments have been made with a 2.25 million crystal which vibrates well and gives the same effects with Elodea as the 1.25 million. A 6 million crystal, 0.45 mm. thick, does not vibrate strongly. We are at present engaged in increasing the frequency to the highest point possible to see how biological effects will vary with the frequency.

A convenient means of finding the resonant frequency of the crystal is to set it up between the two tin foil electrodes with holes in their centers (to allow light to pass for microscopic observation) and then place a drop of water on the crystal. At various settings of the condenser the water will be violently agitated and broken up into fine droplets like steam. Low melting point crystals placed in the water show that the temperature does not rise but that the "steam" is mechanically formed, as observed in various ways by Wood and Loomis (1), and not a condensation from vapor. The exact specifications for an oscillator giving various frequencies will be found as an appendix to this paper.

If an Elodea leaf covered with a cover slip is mounted on a crystal whose resonant frequency is 400 kilocycles, and relatively weak (by reducing filament current) sound waves sent through the leaf, it can be observed under the microscope that only certain areas in the leaf show the characteristic whirling of the chloroplasts described in our previous paper (2). The areas do not correspond to any position on the crystal but to some peculiarity in the leaf, as moving a leaf to a new position over the crystal does not necessarily change the areas of marked whirling. These areas of whirling are most marked where air bubbles, which vibrate strongly, are caught under the leaf and where the cells are several layers in thickness, near the midrib (which also contains

air in intercellular spaces). Part at least of the condition for rapid whirling is the distance of the leaf from the crystal. By attaching the coverslip to a mechanical device for adjusting its distance from the crystal, the amount of water between coverslip and crystal can be varied and a slight change in this layer of water will cause whirling in a given area to start or to stop. These effects are no doubt due to interference of two sets of sound waves resulting in complicated interference patterns with nodes and internodes. Fine particles like red blood corpuscles suspended between crystal and coverslip can be observed to collect in nodes forming such a pattern. The chloroplasts in Elodea cells cannot do so since they are restricted in movement by the cell walls but in a region which happens to be an internode, they will undergo rapid whirling movements. The part played by an air bubble in causing rapid whirling is no doubt to offer a reflecting surface around which interference pattern and nodes appear. The whirling itself is probably due to the radiation pressure of the sound waves as they pass through the cells.

Another phenomenon regularly observed is a variation in the rate and character of the whirling as the variable condenser is changed to vary the frequency. For instance, over a range of 10 kilocycles, there appeared maximum whirling in a given area of the leaf at 407, 409, 410.4, 412.5, 415, and 417 kilocycles, *i.e.* a maximum approximately every 2 kilocycles, with no whirling or very slow whirling between.

In order to understand the changes in whirling motion imparted to the biological material placed upon the quartz as the frequency is varied, it is necessary to digress a moment and consider the forces acting upon an oscillating quartz disk. As is well known, a natural quartz crystal has three electric axes perpendicular to the optic axis. (See Fig. 1.)

The disk is cut as indicated by the shaded portion, *i.e.* so that one of the electric axes shall be perpendicular to the plane of the disk. If pressure is applied to the side of the disk corresponding to $A-$ a negative charge will accumulate there, while correspondingly if a negative charge is applied there without pressure the disk will contract as if the equivalent pressure had been applied. The same holds true with positive charges on the $A+$ side. On the other hand, when a positive charge is placed on the nega-

tive side and a negative charge on the positive side, the crystal will expand. A rapid alternation of charges causes the crystal to oscillate and as a first approximation the crystal can be considered to be an oscillating rigid piston. This would be rigorously correct if the disk were perfect and infinitely large but with a finite disk the forces are not symmetrical near the edges and a complex wave pattern is formed in the crystal. This can easily be seen by first considering a point O on the surface of the crystal near the center (Fig. 2). If a unit negative charge is placed on the under surface with the corresponding positive charge on the
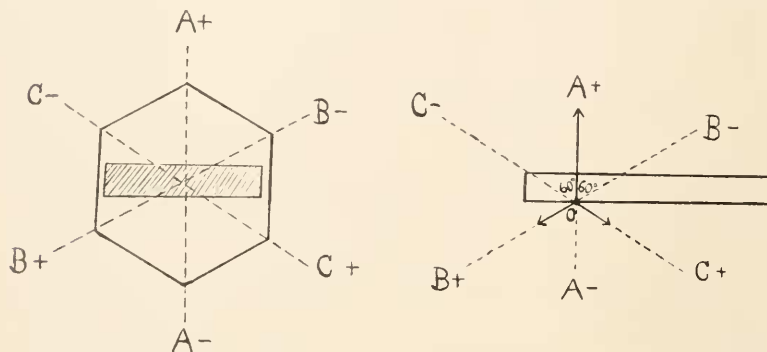
Fig. 1. Quartz crystal (shaded) cut perpendicular to optic axis. Electric axes indicated by AA, BB, CC.

Fig. 2. Vectors showing forces in point O in crystal.

upper, the crystal at "O" will tend to contract along the axis $OA+$ and expand along the axes $OB-$ and $OC-$. The intensities of these forces are directly proportional to the potential gradiants along the respective axis. The forces along $OB-$ and $OC-$ are therefore only half as great as along $OA-$ since the distances through the crystal along these axis are twice as great as along the axis $OA-$ (the angles between the axis being 60°). The vector resolution of the forces $OC-$ and $OB-$ along $OA-$ shows that they are equivalent to a force opposed to the force along $OA-$ and of magnitude equal to one half that of $OA-$. The vector equivalent of all three forces is therefore a single force along $OA-$ equal to half of what that force would be if the forces along the axes $OC-$ and $OB-$ were not present.

This symmetry does not maintain however near the edges of the disk. Consider the point Q, Fig. 3. The axis QB is not in

the crystal at all.   The resolution of the forces along $QA-$ and $QC-$ gives a force along QX equal to the force along $QA+$ multiplied by $\frac{1}{2}\sqrt{3}$.   It is clear, therefore, that the forces near the edges are not symmetrical and tend to produce distortions which travel in waves across the disk.

A second system of forces are also acting on the disk.   As the quartz contracts normally to the surface it expands parallel to the surface (this effect is best seen in a rectangular plate).   Thus the series of longitudinal waves create interference patterns with the traverse waves.
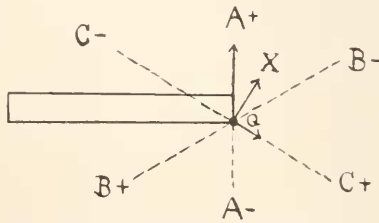
FIG. 3.   Vectors showing forces in point Q in crystal.

Thirdly, it has been shown that even with a perfect quartz crystal the intensity of the piezo electric effect varies in different parts of the crystal.   Dye has photographed the distortions produced in the interference fringes of an interferometer when one of the plates is an oscillating quartz disk.   These photographs show most beautiful and complex patterns which showly drift across the plate when the frequency is slowly changed.

Fortunately in biological investigations under the microscope good use can be made of these complex patterns.   Thus, without changing the position of the specimen on the crystal, one can by merely changing slightly the frequency, cause these patterns to shift so that any particular part of the specimen can be made to experience forces of varying magnitude and direction.   Thus in a particular cell of *Elodea* the chloroplasts can, at will, be made to rotate slowly or rapidly, clockwise, or counter clockwise, in one vortex or in a series of vortices, while merely watching the specimens under the microscope and observing the effects produced as the frequency is slowly varied.

By increasing the intensity, the leaf of *Elodea* can be agitated so violently that the chloroplasts themselves are broken up into a

fine green emulsion which completely fills the cell.   This effect is
not due to heating, since crystals of ethyl stearate, melting at
30–31° C. and placed on the *Elodea* leaf, are not melted even after
15 minutes, nor does slowly heating *Elodea* leaves bring about this
effect.   Neither is it due to possible mechanical rupture of the
cellulose wall or mixing of the vacular sap with the chloroplasts,
since unrayed cells can be crushed with a needle and their chloro-
plasts do not break up in this characteristic manner.   The emulsi-
fication is caused by the tearing action of the sound waves.

Perhaps it should be emphasized at this point that these effects
are all due to high frequency sound waves and not to any influence
of the oscillating electrical field, as control experiments using
glass plates of a size similar to and replacing that of the quartz
crystal have shown.

Some of the more interesting of the effects observed with the
spectacle lens crystal and frequencies of 1,250 kilocycles are as
follows:

Amœbæ proteus or *dubia*,[1] moving along the surface of the
crystal are not particularly affected by an intensity that causes the
inclusions in small vacuoles of the *Amœba* to rotate on their axes.
Higher intensities cause a mild whirling of the more liquid regions
of the *Amœba* followed by rupture of the pellicle on one side and
extrusion of the contents which join the general whirl of fluid in
the medium.   There is a tendency for the *Amœba* to move more
rapidly during the raying as if the endoplasm became more
liquid.   After this there is a sudden change in direction of move-
ment.

Both unfertilized and fertilized sea urchin (*Arbacia*) and star-
fish egg are violently agitated and may spin around.   The jelly
is torn off and the fertilization membrane may be broken.   The
eggs are thrown into rows or clumps and eventually cytolyze
either partially or completely, the cytolysis taking place on one
side and sometimes within the fertilization membrane.   There is
no movement of materials inside the egg caused by raying, as can
be determined with certainty by using centrifuged eggs, the
stratified layers remaining intact until cytolysis takes place.
Cytolysis may take place from any of the stratified layers.   How-
ever, if the unfertilized centrigufed eggs are placed in diluted sea
water (40 distilled water to 60 sea water) and thus made less

viscous, the inside may be made to whirl. The whirling takes place in the lighter layers, including the oil, clear and granular layers, but only along the edge of the pigment layer, most of which remains intact. The oil drops tend to remain together, but the clear and granular layers become mixed and after ten or fifteen minutes of whirling a clear zone can not be distinguished. The direction of rotation may be reversed instantly by a slight change in frequency. Sometimes instead of a whirling of the protoplasm, there is a streaming of granules similar to that of an *Amœba*. No whirling of protoplasm nor movement of granules has been observed in eggs put in dilute sea water and not centrifuged. This may be due to the fact that the less dense material, where the whirling takes place is not separated out from the heavier pigment granules.

The asters are quite unaffected by raying. Cleavage furrows will come in normally during raying, even when the egg is violently agitated. When an egg has been slightly cytolyzed by raying we have observed that the furrow may come in at the proper place. Eggs in the two or four cell stage may have one or two blastomeres cytolyzed and the others unaffected.

Arbacia plutei swimming slowly are paralyzed by a momentary raying, presumably because the cilia are torn off. Otherwise they look uninjured but more prolonged treatment or greater intensity will tear them to pieces, leaving only fragments of the skeleton behind.

The gill cilia of *Mytilius* do not seem to be affected by violent agitation of the sea water about them, until the cilia and gill filaments are actually torn to pieces.

Pigment cells well-expanded in the scales of *Fundulus* are not affected, although the scales are rapidly agitated as the waves impinge upon them.

Frog abdominal muscles mounted on the crystal show no contraction or movement although air bubbles and blood corpuscles on top of the muscle tissue whirl rapidly. The waves must have passed through the muscle tissue to reach the air bubbles and corpuscles.

Fragments of the rays of the ctenophore, *Mnemiopsis*, containing luminous material, mounted on the crystal in the dark and waves passed through, are agitated and occasionally

luminesce. There is no continual luminescence which can be attributed to the waves but only the sporadic luminescence connected with sudden movement of the fragment such as can be obtained on jarring the table containing fragments of *Mnemiopsis,* even when not exposed to high frequency sound waves.

Fundulus embryos within the egg, with beating hearts, subjected to waves of an intensity to agitate the eggs but not so great an intensity as to interfere with observation of the heart beat show no marked effect upon the character of the beat or circulation. In fact only the effect observed was a slight increase in rate during raying which can be accounted for by a slight increase in temperature, that undoubtedly occurs when these high frequency waves carrying considerable energy, are absorbed by the medium. The embryos were rayed 1 minute and then not rayed for one minute while the heart beats were counted. In four experiments the rates were: Rayed—148, 157, 140, 132; unrayed —140, 148, 122, 122, respectively. The average increase in rate was about 8 per cent., which can be accounted for from the known effect of temperature on the heart beat of *Fundulus heteroclitus,*[2] by a rise of temperature from 22° C. to about 23° C.

Perhaps it should be emphasized again from the experiments on muscle, heart, luminous cells and chromatophores that there is no stimulating effect of these waves similar to the stimulation by electrical or sudden mechanical disturbance.

Fertilized *Fundulus* eggs mounted on the crystal can be very violently agitated and the oil drops and granules within made to dance. The yolk can be thoroughly stirred and the surface of the protoplasm can be observed to move and bend. Dr. Elmer Butler has carried these eggs to the point of hatching and finds the development and the embryos normal. If the agitation has continued so long as to burst the protoplasmic surface development does not proceed. An intensity of raying which does not destroy the surface has no effect on development while a slightly greater intensity results in dissolution and cytolysis.

Study of a large number of cells and tissues, some of which are recorded above, has led us to the conclusion that the effects of these waves, apart from slight heating, are purely mechanical. If intense enough, practically all cells can be cytolyzed. It is as

if one could grasp a cell in both hands and bend it violently back and forth at a very rapid rate. Delicate structures on the outside of a cell are torn off. If the cell is very small it is thrown into nodes so quickly as to escape injury. If the cell can be held fixed and is not too viscous, its contents can often be made to whirl before it breaks down.

From the whirling one can gain an idea of the viscosity of the cell contents. Perhaps the chief value of the waves for biological investigation lies in the evidence obtained from their action regarding the viscosity of cells. It should be emphasized, however, that comparative studies of viscosity are difficult because of the great complexity of the sound wave patterns under the cover slip, both horizontally and vertically. Two cells in different portions of the same microscopic field are not necessarily exposed to the same radiational forces and great caution must be used in drawing conclusions regarding viscosity or resistance to tearing by difference in behavior of cells.

High frequency sound waves offer a new means of affecting the interior of cells without necessarily breaking down the cell wall. They will be of most value when a beam of given frequency and controlled intensity can be sent through a cell or tissue in a particular direction.

APPENDIX.

For those biologists who desire to construct a low-powered oscillator, the following constructional details ought to suffice.

The following apparatus is recommended.

One No. 852 Radiotron 75 watt tube,

One tube holder

One filament transformer to give 10 volts

One plate transformer to give 2,000 volts

One 5,000 ohm resistance

Several transmitting condensors (designed to withstand 5,000 volts) with an aggregate capacity of about 0.1 microfarad

One rheostat

Some heavy copper strip to wind the inductance

Some fine wire to make the secondary

All of the above can be bought from any radio store carrying

parts for transmitting sets, and should not cost more than $100 in the aggregate.

Fig. 4 shows the wiring diagram and a suggested arrangement of the parts. The iron of the transformers should be on the side of the tube away from the oscillating parts and should be at least a foot from the tube. All the parts can conveniently be mounted on a board 30 x 10 inches.
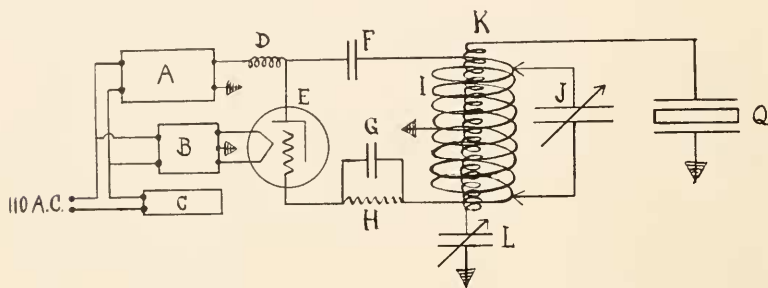
FIG. 4.   Constructional diagram for a 75 watt oscillator.   A, plate transformer; B, filament transformer; C, rheostat; D, choke coil; E, Radiotron No. 852; F, Blocking condenser. G, Grid leak condenser. H, Grid lead. I, Inductance coil; J, tuning condenser; K, secondary coil; L, Variable condenser; Q, Quartz plate between electrodes.

The rheostat should be mounted in the lead from the 110-volt A. C. house circuit and can be used to regulate the voltage. The primaries of the transformers should be connected in parallel across the house circuit. One side of the secondary of the plate transformer should be connected to the center tap of the filament transformer which point should also be grounded. The other side of the secondary should go through a choke coil to the plate. The choke coil can be made by winding about 100 turns of fine wire on a bakelite tube one or two inches in diameter.

The inductance can be made by winding fifteen or twenty turns of heavy copper wire on a bakelite tube six or eight inches in diameter. The plate should be connected to one tap on the inductance through a blocking condenser of about .002 microfarad capacity. The grid should be connected to the other tap on the inductance through a by-pass condenser of about the same capacity and a grid leak of above 5,000 ohms resistance. The center tap of the inductance should be grounded. The secondary can be made by winding 100 turns of fine wire on a bakelite tube

which can be slipped inside the primary inductance. One end of the secondary should go to one plate of the crystal holder (the other plate of the holder being grounded). The other end of the secondary should be connected to ground through a variable condenser or to a rod of metal perhaps 1 inch diameter and ten inches long, which is not grounded.

The quartz crystal need not be larger than one square inch. It should be cut perpendicular to an electric axis. Its natural frequency of oscillation will depend on its thickness.

Mm. Thick.	Frequency (Approx.).
1	2,900,000 cycles per sec.
2	1,450,000 " " "
3	966,000 " " "
4	725,000 " " "
5	580,000 " " "
etc.	etc.

The oscillating circuit should be tuned to approximately the frequency of the crystal.

The crystal holder can conveniently be made out of two microscope slides and two thin brass strips with holes cut in them for use with the microscope. The microscope should be at least three feet from the oscillator so that movements of the operators body shall not change the frequency. The high tension lead to the microscope should be shielded by surrounding it with a grounded metal tube and the microscope itself should be grounded to prevent small spark discharges to the observer.

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[1] Kindly supplied by Dr. J. A. Dawson of Harvard University.
[2] Unpublished data of Dr. Otto Glaser of Amherst College.

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